UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: October 18, 2018

SUBJECT: Bacteriophage active against *Xylella fastidiosa* (PC Code 116404) – Human Health Assessment Summary

Decision Numbers:	527226, 527227, 527288, 527226, 527227
DP Numbers:	444193, 444194, 444202, 448255, 448257
EPA File Symbols:	92918-E (MP) and 92918-R (EP)
Active Ingredient Type:	Microbial
PC Code:	116404
Active Ingredient Tolerance/Exemption:	7F8562
MRID Numbers:	501593-04, 501593-05, 501593-06, 501593-07, 501593-08, 503641-01, 504774-01, 506188-01, 506188-02 and 506188-03

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TO: Alex Boukedes, Risk Manager
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Background:

In March of 2017, EPA received an application from Otsuka Pharmaceutical Co., Ltd. for Section 3 registration of a technical product OPC-721 (File Symbol 92918-E) containing Bacteriophage active against *Xylella fastidiosa* (0.00028%). This submission also includes an application for the end-use product XylPhi-PD (File Symbol 92918-R), which contains Bacteriophage active against *Xylella fastidiosa* (0.00028%) as the sole active ingredient. Bacteriophage active against *Xylella fastidiosa* are naturally occurring organisms and are typically isolated from plant tissue infected with the *Xylella* or *Xanthomonas* bacteria. The target of these bacteriophage, *Xylella fastidiosa*, is a bacterium which causes Pierce's Disease in grapevines. Bacteriophage active against *Xylella fastidiosa* infect these disease-causing bacteria with a high degree of specificity, replicate and cause cell lysis, thereby killing the bacteria and releasing additional bacteriophage. This process, known as the lytic cycle, will continue until the host bacteria (*Xylella fastidiosa*) populations are greatly reduced or eliminated. Grapevine infection with *Xylella fastidiosa* bacteria (Pierce's Disease) significantly inhibits the ability of grapevines to produce fruit and was first noted in California in the late 1800s.

Microbial Use Pattern:

Bacteriophage active against *Xylella fastidiosa* can be used for preventative action to protect growing vines or as a curative treatment after the symptoms of Pierce's Disease are present. The end-use product is applied by injecting 40 to 120 microliters directly into the vascular system of grapevines at a depth ranging from 2-4 mm, depending on the diameter of the plant stem tissue. Injections are made using a pressurized injection device capable of delivering the dose accurately. Injection applications, which are typically made 2-3 times during the growing season, include a primary treatment 6-8 weeks after vines are flush from dormancy and secondary treatment 4-6 weeks after the primary treatment. An additional treatment may be made anytime thereafter depending on the disease pressure.

Product Chemistry:

The Product Identity, Manufacturing Process, Discussion of Formation of Unintentional Ingredients, Analysis of Samples, Certification of Limits, Storage Stability/Corrosion Characteristics and Physical/Chemical Characteristics data submitted for these products are acceptable. These studies are available under MRID numbers 506188-01 through 506188-03, 501593-01, 501593-02, 503233-01 and 503489-01 (Ref. 2).

Human Health Assessment:

The toxicity, pathogenicity and infectivity data (Ref. 1) are summarized below for the manufacturing-use (technical) product, OPC-721 (Table 1A and 1B) and the end-use product, XylPhi-PD (Table 1B). In acute oral and dermal toxicity studies performed with rats, high doses of Bacteriophage active against *Xylella fastidiosa* were administered and revealed no adverse effects or mortalities, demonstrating a lack of acute mammalian toxicity by these routes. In addition, acute dermal and eye irritation studies in rabbits revealed no significant irritation reactions to the test substance. Note that the 870 Series acute toxicity studies were performed with OPC-721 rather than the end-use product, however use of this test material is considered acceptable to support/represent the end-use formulation as well. The supporting information/data

provided is sufficient to satisfy the Tier I toxicology data requirements for human health risk assessment of Bacteriophage active against *Xylella fastidiosa* and the associated end-use product.

Table 1A. Summary of Toxicity, Pathogenicity and Infectivity Data Supporting the Manufacturing-Use Product, OPC-721

Data Requirement	OCSPP Guideline No.	Results Summary, Classification and Toxicity Category	MRID No.
Acute Oral Toxicity/Pathogenicity Acute Pulmonary Toxicity/Pathogenicity Acute Injection Toxicity/Pathogenicity	885.3050 885.3150 885.3200	The Acute Oral Toxicity/Pathogenicity, Acute Pulmonary Toxicity/Pathogenicity and Acute Injection Toxicity/Pathogenicity studies were waived based on the following: 1) The purpose of the Toxicity and Pathogenicity series 885.3050, 885.3150 and 885.3200 guidelines for microbes is to test animals for potential infectivity and pathogenesis of an active ingredient at a maximum hazard dose for potential infectivity and pathogenesis of an active ingredient. Since bacteriophage are obligate intracellular parasites of prokaryotes and have no ability to replicate in mammalian cells, there is no utility in testing for any overt toxicity, infectivity and pathogenesis of a bacteriophage active ingredient in animals. 2) Bacteriophage are ubiquitous biological organisms present in soils, water and in or on most foods. Only rarely are bacteriophage associated with negative effects, most commonly involving carriage of DNA between bacterial cells by temperate phage. Only lytic bacteriophage are utilized in these products, confirmed in culture and with whole genome sequencing and analysis. Each isolate is analyzed to confirm they are lytic and not able to integrate into a host genome and to rule out carriage of any extraneous DNA between cells, minimizing any concern of off-target effects. **Classification: Acceptable**	503641-01
Hypersensitivity Incidents	885.3400	Waiver requested for hypersensitivity incidents under 885.3400. The waiver request was granted by the Agency due in part because guideline 885.3400 is not a testing requirement of registration but rather an ongoing duty to report any incidents of Hypersensitivity related to pesticide use "including immediate type and delayed-type reactions of humans or domestic animals, [that] occur during the testing or production of the TGAI, MP, or EP, or are otherwise known to the applicant must be reported if they occur" under FIFRA 6(a)(2) adverse effects reporting procedures. **Classification: Acceptable**	504774-01

Table 1A. Summary of Toxicity, Pathogenicity and Infectivity Data Supporting the Manufacturing-Use Product, OPC-721			
Data Requirement	**OCSPP Guideline No.**	**Results Summary, Classification and Toxicity Category**	**MRID No.**
Cell Culture	885.3500	The purpose of the Cell Culture guideline, specific for any pesticidal viruses, is to test in mammalian cell lines for potential infectivity and pathogenesis of virus active ingredients. Bacteriophage as a group are obligate intracellular parasites of prokaryotes and have no ability to replicate in mammalian cells or cell lines. In particular, the bacteriophage are specific to the prokaryotic type IV pilus of *Xylella* and *Xanthomonas* species and cannot infect a cell lacking this phenotype. Further, only lytic bacteriophage are utilized in these products, confirmed in culture and with whole genome sequencing and analysis. Each isolate is analyzed to confirm they are lytic and not able to integrate into a host genome and to rule out carriage of any extraneous DNA between cells. **Classification: Acceptable**	504774-01

Table 1B. Summary of Acute Toxicity Data Supporting OPC-721 and the End-Use Product, XylPhi-PD			
Data Requirement	**OCSPP Guideline No.**	**Results Summary, Classification and Toxicity Category**	**MRID No.**
Acute Oral Toxicity	870.1100	A group of five fasted female 8-week-old rats were given a single oral gavage dose of 5,000 mg/kg bw of undiluted OPC-721. Dosing was on Day 0, and the animals were observed for up to 14 days. No toxicity was observed in the female rat after exposure to a single oral dose of 5,000 mg/kg bw. There were no deaths, abnormal clinical signs, or abnormal gross necropsy findings, and all of the animals gained weight during both weeks of the study. **Toxicity Category: IV** **Classification: Acceptable**	501593-04

Table 1B. Summary of Acute Toxicity Data Supporting OPC-721 and the End-Use Product, XylPhi-PD

Data Requirement	OCSPP Guideline No.	Results Summary, Classification and Toxicity Category	MRID No.
Acute Dermal Toxicity	870.1200	Five male and five female 8-week-old rats were dermally exposed to undiluted OPC-721 applied to clipped application sites comprising approximately 10% of the body surface area for 24 hours at a dose level of 5,000 mg/kg bw. The animals were treated on day 0 and observed for 14 days. No toxicity was observed in the rat after exposure to a single dose of 5,000 mg/kg bw. There were no deaths or treatment-related gross necropsy findings, and all of the animals gained weight during both weeks of the study. **Toxicity Category: IV** **Classification: Acceptable**	501593-05
Acute Inhalation Toxicity	870.1300	The acute inhalation toxicity study requirement was waived based on the following rationale: Bacteriophage are ubiquitous biological organisms present in soils, water and in or on most food. Only rarely are bacteriophage associated with negative effects, most commonly involving carriage of DNA between bacterial cells by temperate phage. Only lytic bacteriophage are utilized in these products, confirmed in culture and with whole genome sequencing and analysis. Each isolate is analyzed to confirm they are lytic and not able to integrate into a host genome and to rule out carriage of any extraneous DNA between cells, minimizing any concern of off-target effects. **Toxicity Category: IV** **Classification: Acceptable**	503641-01
Acute Eye Irritation	870.2400	The irritant and/or corrosive effects of Bacteriophage active against *Xylella fastidiosa* was determined in rabbits (N=3) with use of a 0.1 mL solution of test article as a single ocular administration. No signs of ocular irritation were observed up to 72-hours after application of the test article. Per the Kay and Calandra Evaluation Criteria, the test article is considered to be a non-irritant to the ocular tissues. **Toxicity Category: IV** **Classification: Acceptable**	501593-06

Table 1B. Summary of Acute Toxicity Data Supporting OPC-721 and the End-Use Product, XylPhi-PD			
Data Requirement	**OCSPP Guideline No.**	**Results Summary, Classification and Toxicity Category**	**MRID No.**
Acute Dermal Irritation	870.2500	Three young adult male New Zealand White rabbits were dermally exposed for four hours to 0.5 mL of undiluted OPC-721 applied to 1-inch by 1-inch clipped application sites. The animals were observed at one hour after patch removal and at 24, 48, and 72 hours after patch application, and the responses were scored according to Draize. There were no observations of erythema, edema, and/or other signs of dermal irritation noted on any application site at any time point during the study. **Toxicity Category: IV** **Classification: Acceptable**	501593-07
Skin Sensitization	870.2600	The dermal sensitization of Bacteriophage active against *Xylella fastidiosa* was evaluated in male and female guinea pigs per the Modified Buehler Design. Test organisms were treated with 100% test article, one per week, for 3 consecutive weeks, followed by a challenge and re-challenge. Based on the results of the study the test article is not expected to be a dermal sensitizer. **Classification: Supplemental** (not a required study)	501593-08

Aggregate Exposure and Risk Characterization:

In examining aggregate exposure, FFDCA section 408 directs EPA to consider available information concerning exposures from the pesticide residue in food and from all other non-occupational exposures. These non-occupational exposures include exposures through drinking water (via ground water or surface water) as well as exposure through pesticide use in gardens, lawns or buildings (residential and other indoor uses). However, since Bacteriophage active against *Xylella fastidiosa* is proposed for agricultural use sites only, residential/non-occupational exposures (other than food and drinking water) resulting from use of these products are not expected.

Food Exposure and Risk Characterization

Based on the proposed uses for Bacteriophage active against *Xylella fastidiosa* (injection application to grapevines), exposure to this active ingredient through food commodities is a possibility. However, Bacteriophage are ubiquitous in the environment and are naturally consumed in food commodities without any known adverse effects. Further, bacteriophage are obligate intracellular parasites of prokaryotes and have no demonstrated ability to replicate within mammalian cells. Based on these characteristics of bacteriophage and the lack of adverse effects in the available toxicity studies performed with Bacteriophage active against *Xylella fastidiosa*, dietary exposure resulting from use of this pesticide is not be expected to pose any quantifiable risk. Therefore, a quantitative food exposure assessment was not performed.

Drinking Water Exposure and Risk Characterization

Based on the proposed uses for Bacteriophage active against *Xylella fastidiosa*, the potential for it to enter surface or ground water does exist. However, exposure to humans from residues of Bacteriophage active against *Xylella fastidiosa* in drinking water is unlikely as the application method (grapevine injection) limits the potential for significant environmental distribution. Further, published literature indicates that humans routinely interact with phages as they are ubiquitous in the natural environment, including in lakes and marine waters (Ref. 3). Nevertheless, should exposure to this active ingredient through drinking water occur, bacteriophage are obligate intracellular parasites of prokaryotes and have no demonstrated ability to replicate within mammalian cells. Based on these characteristics and the lack of adverse effects in the acute toxicity studies performed with Bacteriophage active against *Xylella fastidiosa*, drinking water exposure resulting from use of this pesticide is not expected to pose any quantifiable risk. Therefore, a quantitative drinking water assessment was not performed.

Non-occupational, Residential Exposure and Risk Characterization

As previously stated, Bacteriophage active against *Xylella fastidiosa* is intended only for agricultural use in grapevines. As a result, residential and non-occupational exposures (other than food and drinking water) resulting from use of this active ingredient are not anticipated.

Cumulative Effects:

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify or revoke a tolerance, EPA consider "available information concerning the cumulative effects of a pesticide's residues and other substances that have a common mechanism of toxicity". Bacteriophage active against *Xylella fastidiosa* is not toxic and does not have a common mechanism of toxicity with other substances. Consequently, FFDCA section 408(b)(2)(D)(v) does not apply.

Infants and Children:

FFDCA section 408(b)(2)(C) provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the database on toxicity and exposure, unless EPA determines based on reliable data that a different margin of safety will be safe for infants and children. This additional margin of safety is commonly referred to as the Food Quality Protection Act Safety Factor. In applying this provision, EPA either retains the default value of 10X or uses a different additional safety factor when reliable data available to EPA support the choice of a different factor. As discussed previously, EPA has concluded that Bacteriophage active against *Xylella fastidiosa* is not toxic, pathogenic, or infective to mammals, including infants and children. Because there are no threshold levels of concern to infants, children, and adults when Bacteriophage active against *Xylella fastidiosa* is used in accordance with label directions and good agricultural practices, EPA concludes that no additional margin of safety is necessary to protect infants and children.

Occupational Exposure and Risk Characterization:

Based on the proposed use pattern of Bacteriophage active against *Xylella fastidiosa*, there is potential for occupational exposure to applicators and handlers through the dermal route. However, the supporting data indicates that there is no significant toxicity, irritation, pathogenicity or other adverse effects attributable to this active ingredient. Further, the product label specifies use of protective eyewear, long-sleeved shirt and long pants, waterproof gloves and shoes plus socks while handling this active ingredient. In addition, though there is only minimal concern for occupational inhalation exposure with this product, all microbial pesticide product labels require use of a dust/mist filtering respirator by handlers. Therefore, since occupational exposure to Bacteriophage active against *Xylella fastidiosa* is not expected to exceed any toxicity thresholds when pesticide handlers follow the precautions and requirements identified on the product label, a quantitative occupational assessment has not been performed.

Risk Characterization:

The Risk Assessment Branch (RAB) has evaluated the hazard and exposure potential resulting from the use of Bacteriophage active against *Xylella fastidiosa* as a pesticide, with consideration given to the relevant safety factors in FFDCA and FIFRA. Based on the rationale provided above, RAB concludes that the submitted data support a finding that no unreasonable adverse effects to the U.S. population in general and to infants and children in particular, will result from the pesticidal uses of Bacteriophage active against *Xylella fastidiosa* when the product label instructions and precautions are followed.

References:

1. Bacteriophages active against *Xylella fastidiosa*; Review of Data Supporting FIFRA Section 3 registration of Bacteriophages active against *Xylella fastidiosa* - Memo Dated 4/18/2018 from Joel Gagliardi [Risk Assessment Branch] to Risk Manager, Alexandra Boukedes [Microbial Pesticides Branch], (Decision Nos: 527226, 527227, 527288).

2. Product Characterization and Toxicity Assessment for FIFRA Section 3 Registration of and MUP and an EP containing Bacteriophages active against *Xylella fastidiosa*; Review of 75-day deficiency letter response - Memo Dated 10/3/2018, from Joel Gagliardi [Risk Assessment Branch] to Risk Manager, Alexandra Boukedes [Microbial Pesticides Branch], (Decision Nos: 527226, 527227).

3. Gill, J., and Young, R. (2011). Therapeutic Applications of Phage Biology: History, Practice, and Recommendations. Chapter 17 in A.A. Miller and P.F. Miller (eds.), *Emerging Trends in Antibacterial Discovery: Answering the Call to Arms.* Caister Academic Press, Norfolk, UK.